Checking whether or not the LT/CT protein stayed connected to the pSB1C3 plasmid (containing GFP) as it arrived at the epithelial lung cell NCLH1650

Steps:

1. using the coloring agent Hoescht to color the plasmid (if a blue fluorescent color appears, the experiment has been successful.) The color gives the DNA a blue color, but does not affect it otherwise in any way.
2. Coloring a clean plasmid (as a reference to the plasmid/protein complex)
    2.1.1. Coloring the plasmid found within the LT complex
3. Connecting the LT-DBD protein to the pSB1C3 plasmid
4. Infecting the lung epithelial cell (NCLH-1650) with the following:
    4.1. LTBD+ pSB1C3
    4.2. CTB+ pSB1C3
5. analyzing fluorescence with the help of the FACS machine, as a way of determining whether or not the plasmid/protein complex has indeed attached itself to the epithelial cells.
6. photographing the cells with a confocal microscope

The next stage, assuming that the protein and plasmid complex have attached to the epithelial cells, is the incubation of the complex with the cells for between 24 and 48 hours and constant checking of the movement of the plasmid towards the nucleus of the epithelial cells. (Assessing movement will be done with the help of a confocal microscope), and the expression of the GFP factor will be assessed as well. (If a green color appears, the DNA has been expressed in the nucleus of the cell.)

Work plan:

1. Coloring the plasmid with the Hoechst agent
2. Mixing 20 micrograms of DNA with 1.1 microliter of color, and incubation at 37 degrees Celsius for 30 minutes. This will be done once with a closed plasmid and once with a cut/opened plasmid.
3. Cleaning the the plasmid of the remaining coloring agent. (The plasmid alone is cleaned with the use of the miniprep kit as written in the protocol, and the plasmid complex is cleaned by way of dialysis as written in the dialysis protocol.)
4. Attaching the LTBD protein to the pSB1C3 plasmid:

- 2000 nanogram of protein are mixed with 5 microgram of DNA for 45 minutes at incubated at 37 degrees Celsius (once with the closed plasmid and once with a plasmid that has been linearized).
- 500,000 epithelial cells will be incubated with the plasmid/protein complex for one hour at 4 degrees Celsius.)
- Following the incubation, the FACS machine will be used to measure fluorescence.
- The cells will be checked/measured with a confocal microscope.